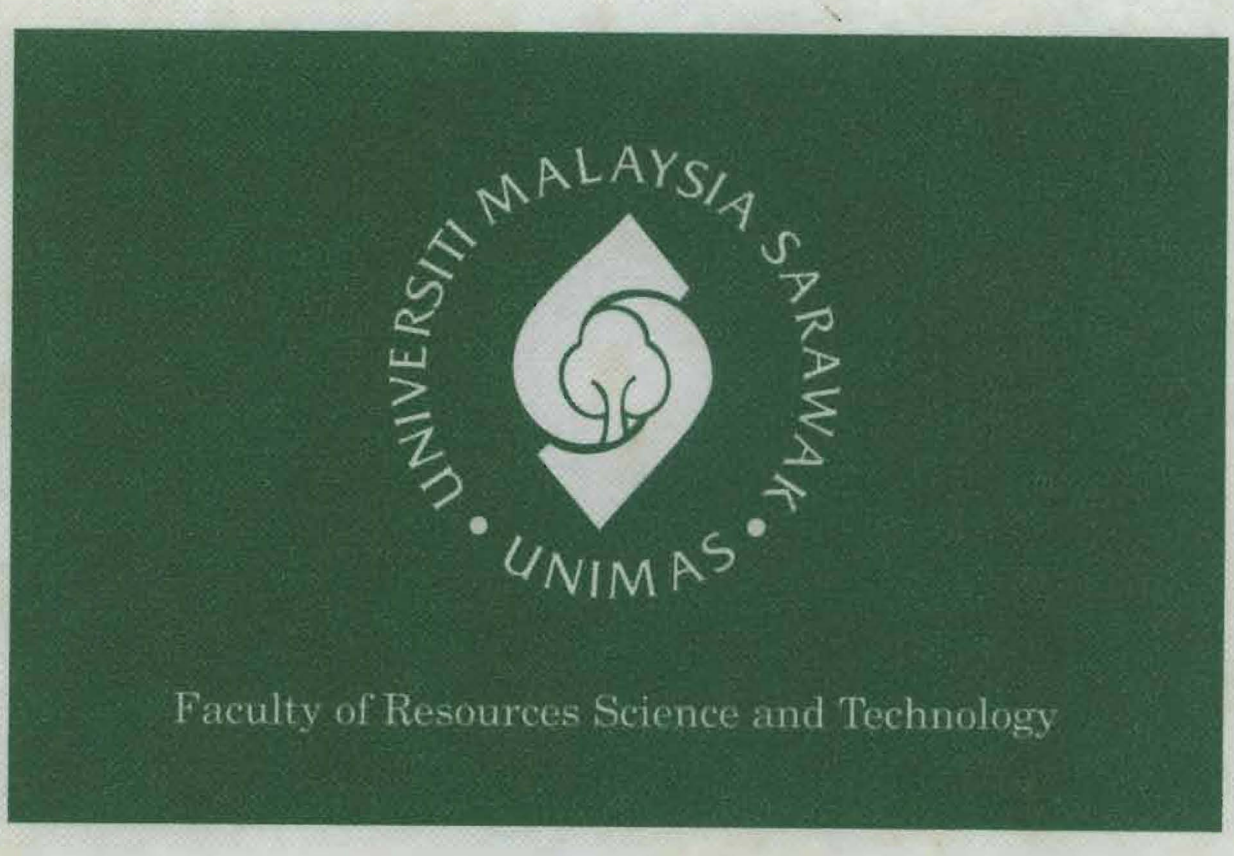

FEASIBILITY ASSESSMENT OF PROCEDURES TO EXTRACT QUALITY TOTAL GENOMIC DNA FROM HAIR SAMPLES OF THE JAMNAPARI GOATS

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Feasibility Assessment of Procedures to Extract Quality Total Genomic DNA from Hair Samples of the Jamnapari Goats

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List of Abbreviations

AGE	Agarose gel electrophoresis
Ca	Calcium
^0C	Degree Celsius
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
H_2O	Water
L	Liter
%	Percent
PCR	Polymerase chain reaction
M	Mole
ml	Mili liter
DNA	Deoxyribonucleic acid
RNA	Dideoxyribonucleic acid
NaCl	Sodium chlodride
SDS	Sodium dodecylsulfate
NaI	Sodium iodide

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Abstract

Jamnapari goat is one of the well-defined breeds of goat in Indian. It is normally bred for milk and meat as the Jamnapari meat is known to contain low cholesterol. As a commercial animal, the genetic study of Jamnapari goat is very vital in order to preserve and improve the quality of the products. In this study, the hair of Jamnapari goat is used as a source to isolate total genomic DNA. The hair sample has many benefits over the bloods and buccal samples: can be stored in room temperature, easy to collect and clean. The main aim of this project is to evaluate the feasibility and efficiency of a laboratory method in the isolation of good quality and quantity genomic DNA from the hair sample of Jamnapari goats. By using the phenol chloroform and salting out method with different parameters, the concentration of extracted DNA was very small and there was no band shown for some samples after agarose electrophoresis. In order to determine the quality and quantity of DNA, all the samples undergo further analysis such as spectrophotometer and polymerase chain reaction (PCR). The spectrophotometric analysis showed that the concentration of extracted DNA was about 0.3-4.5μg/μl with contanmaination, while the amplification using PCR approach at region of *cytochrome b* was able to produce the product with size range of 359bp. Further analysis of the DNA sequences of this amplified DNA showed that approximately 99% of the generated sequences were identical with the region of *cytochrome b* for the Jamnapari goats (*Capra hircus*). The results showed that although the procedure of DNA extraction produces very low amount of DNA, the methods yielded DNA that can be used for further analysis such as PCR amplification and DNA sequencing.

Key words: Hair DNA, phenol chloroform method, salting out method, polymerase chain reaction.

Abstrak

Kambing Jamnapari merupakan salah satu ternakan yang terkenal di India kerana ia mempunyai susu dan daging yang berkolesterol rendah. Sebagai haiwan komersial, kajian tentang genetik bagi kambing Jamnapari sangat penting demi meningkatkan kualiti dan kuantiti produk. Dalam kajian ini, DNA telah diestrak daripada rambut kambing Jamnapari memandangkan sampel rambut mempunyai banyak manfaat berbanding dengan sampel darah dan bukal seperti boleh disimpan dalam suhu bilik, mudah dikumpul dan dibersihkan. Tujuan utama projek ini adalah menilai kecekapan teknik- teknik tersebut dalam pengestrakkan DNA daripada sampel rambut kambing Jamnapari. Dengan menggunakan kaedah fenol kloroform dan pangasingan keluar, kandungan DNA yang telah diestrakkan adalah sangat kecil dan beberapa sampel gagal memaparkan band selepas elektroforesis agarose. Demi menentukan kualiti dan kuantiti DNA, semua sampel mangalami analisis yang lebih lanjut seperti spektrofototmeter dan PCR. Analisis spektrofotometri menunjukkan lebih kurang 0.3-4.5µg/µl DNA telah diekstrakkan, manakala cytocrome b telah digunakan dalam proses PCR dan produk yang bersaiz 359bp telah dihasilkan. Sekuensing DNA juga menunjukkan bahawa 99% DNA yang telah diekstrakkan merupakan milik kambing Jamnapari (Capra hircus). Kesimpulannya, walaupun teknik-teknik pengestrakkan DNA berjaya mnghasilkan DNA yang berjumlah kecil, tetapi DNA yang telah diekstrakkan dapat digunakan untuk analisis yang lebih lanjut seperti PCR dan sekuensing DNA.

Kata kunci: DNA rambut, kaedah fenol kloroform, kaedah pengasingan keluar, PCR

1.0 Introduction

1.1 Jamnapari Goat

Goats are recognized as one of the important species for the world diversity in livestock and play useful role in sustainable agriculture. Indian has about 20 well-defined breeds of goats, which represent a wide spectrum of genetic variability.

One of the well defined breeds of goat is Jamnapari goat, a dual purpose (milk and meat) goat breed. It is also known as Indian Jamunapari, which is one of the ancestors of the American Nubian. It is mainly found in the undulated land of Chakarnagar between the ravines of Jumuna (Yamuna) and Chambal River in Etawah district of Uttar Pradesh, India. The name is derived from the location of the breed beyond the river Jamuna. (Gour *et al.*, 2005)

Jamnapari goat is famous for its large and graceful body. It has long, flat, drooping ears and thick fur on rumps. It also has strongly arched Roman nose and short, flat horns. Besides, it has long, wide pendulous ears and parrot mouth. Male Jamnapari is weighted up to 120 kg while the female is about 90 kg. (FAO, 1982)

Jamnapari goat is also well known in the milk production as it can produce the highest amounts of milk among all Indian goat breeds (Gour *et al., 2005).* According to the FAO (1982), Jamnapari can produce about eight pounds of milk daily and average lactation yields of 2.5 pounds per day. Milk yields increase up to the end of two months and then start to decline for average lactation lengths of 260 days. Besides the milk, it also provides meat for our daily life. The average of meat weight is shown in the Table 1 (in Appendix).

However, the Jamnapari breed is showing endangeredment trend in its native breeding region due to the ecosystem and market demands. Food and Agriculture Organization (FAO)

of the United Nations indicated that approximately 30% of the world's farm animal breeds are at the risk of extinction (FAO, 1997). The officials of the Animal Husbandry Department of Uttar Pradesh stated that the total number of pure-bred Jamnapari does not exceed 5000 (FAO, 1982). This shows that the conservation, multiplication and further improvement of the breed are needed for this small numbers of Jamnapari.

Hence, one of the aims of this study was to assess the feasibility of a laboratory method in isolating a genomic DNA from hair samples of Jamnapari goats, which can help in the evaluation of genetic variability. The genetic variation of this animal is the basic material that can be used for changing the genetic makeup or genetic potentiality of domestic species to suit our needs. By this way, the milk productivity and quality in goat genetic resources can be improved.

1.2 Objectives

1. To extract good quality and quantity of DNA from the hair samples of the Jamnapari goats.
2. To evaluate the feasibility of conventional phenol-chloroform and salting out method with different parameters in the isolation of total genomic DNA from the hair samples of Jamnapari goats.
3. To determine the quality and quantity of the extracted DNA from hair samples.
4. To determine the most optimal method and parameter in the isolation of hair DNA.

2.0 Literature Review

2.1 Genotyping of Livestock

In agriculture, the genome research in animals has progressed rapidly. It is moving from rudimentary genome maps to trait maps and to the gene discovery.

In the genome research of goats, Vaiman *et al.* (1996) reported a first genetic linkage map of the goat genome, which was established by using the cattle and sheep microsatelites. This map consisted of 219 markers, of which 204 markers were incorporated into linkage groups and 15 were mapped by the fluorescence in situ hybridization (FISH). The study showed that there are 55 polymorphic markers can be used and defined a panel of 223 microsatellites which is suitable for goat. Besides that, a linkage map of goat genome was also generated.

Pariset *et al.* (2009) showed the recent evolutionary history of domestic goats by using 26 single nucleotide polymorphism (SNP) for a total of 12896 genotyped assayed, which revealed that the breeds were not similar in terms of genetic variability. Besides that, the Mantel test also showed that there were correlation between genetic diversity and geographic distance.

In order to improve the goat breeds towards economic issues, Othman and Ahmed, (2006) used forty-five animals belong to four Egyptian goat breeds to analyse the CSN1 S2 gene, which is characterized by seven alleles (A, B, C, D, E, F and 0) by using PCR. The results showed that homozygous genotypes AA and BB were observed at frequencies 28.9% and 26.7%, respectively, while the heterozygous genotype AB was displayed in 18 animals (40%) whereas the genotype AF was present in two animals (4.4%). The alleles C, D, E and 0 were not observed in the tested animals. This study is very important as the increase of

homozygous genotypes with high casein content is needed in milk industry. This can do by selecting the animals through molecular analysis.

In addition, Luikart *et al.*, (1999) carried out a study about the parentage testing in goats by using power of 22 microsatellite markers in fluorescent multiplexes. In this case, the multiplexes provide a very high power of individual identification as the probility of finding in two identical genotypes for the 22 loci is smaller than 1 in 1.10^{15i} in each breeds (Mongolian Native Cashmere, Turkish Angora, Swiss Saanen and Spanish Murciana-Grenadina). This research is very useful for the study of population structure, history and diversity in goats. The results can be used in the genetic improvement of domestic breeds.

2.2 Hair DNA

Recently, the isolation of DNA from the hair samples is important in forensic science. A study has been done to confirm that the hair samples contain of approximately 0.4ng of double-stranded DNA by using the Pico-green assay (Heywood *et al.*, 2002).

Hair has many benefits over bloods and buccal samples. According to Leanza *et al.* (2007), hair is easy to collect, does not require costly kits and can be stored at room temperature. The keratin in hair can also protect the DNA from the environment affect (Gilbert cited, 2007). It is a good source as the DNA in bones and muscle usually degrades and becomes contaminated with genetic material from other sources such as bacteria (Schuster, 2007). Besides, the hair samples are also easily be cleaned from the environmental contaminants without affecting the genetic material in the hair (Gilbert, 2007)

However, Leanza et al. (2007) reported that the quantity of isolated DNA from the hair sample was extremely low, which was ranged from 0 to 1.9 μg with average yield of

0.22 (±0.34 μg). This showed that the amount of isolated DNA from hair shaft sample was extremely low to quantify. The isolation of DNA from hair samples is also quite hard as the level of DNA in the tip-end hair is less than the level in the roots (Heywood *et al.*, 2002).

Besides that, the quality of the product might also be affected by the contamination with pigments and other substances, such as RNA, melanine and so on (Leanza *et al.*, 2007). The degree of contamination depends on hair colouration and species. Sometimes, the reagents that used in the extraction of DNA may also inhibit the PCR process such as phenol or Chelex resin left with the extracted DNA (Figarelli, 1999). Hence, this study is done to evaluate the feasibility of a laboratory method to isolate good quality and quantity of total genomic DNA from the hair samples of Jamnapari goats.

2.3 Methods of Extracting Hair DNA

The nature of the wide variety of substrates on which biological samples are deposited can create problems for the DNA analyst. The inhibitors present in the sample substrate will cause the decrease of efficiency in the amplification of samples. The presence of inhibitor can also result in loss of data. Therefore, DNA extraction methods have been developed to eliminate proteins and other inhibitors from the DNA molecules. In addition, the quality and quantity of genome DNA often need to be done before proceed to further analysis using agarose gel electrophoresis and spectrophotometer.

Organic extraction, also known as phenol-chloroform extraction is one of the primary techniques used in isolation of DNA. It has been in use for the longest period of time. In this method, the sample is cut into small pieces. The sodium dodecyl sulfate (SDS) and Proteinase K are added to break the cell walls and proteins that protect the DNA molecules. Then, phenol/chloroform mixture is added to separate proteins from DNA. Normally, the DNA is

more soluble in aqueous portion of the mixture. Hence, the protein and other substances can be separate from the DNA after centrifugation. (Butler, 2005) This method is better for the isolation of high molecular weight DNA and cleans the DNA more thoroughly than Chelex extraction. The extracted DNA is further purified and concentrated by using special filters, producing pure DNA for use in either RFLP or PCR-based analysis. (Rudin and Inman, 2002) However, this organic method is time consuming and involves the use of hazardous chemical reagents. It is also increase the risk of contamination as the sample is required to be transferred multiple tubes. (Butler, 2005) Furthermore, toxic and corrosive organic solvents (phenol- chloroform) are used in most method (Mohammadi *et al.*, 2009).

Since the phenol-chloroform is toxic, it is important to create an efficient DNA extraction procedure, which does not use such materials. Therefore, in this study, the salting-out method is used to isolate the total genomic DNA from hair samples. This method is relatively easy and convenient which involves the preferential hydrolysis and precipitation of cellular proteins (McClintock, 2008). Since the phenol-chloroform is very toxic, the protein and other contaminants are precipitated from the cell lysate by using high concentration of salt such as sodium chloride and potassium acetate. Then, the DNA is recovered by alcohol precipitation. This method may be inefficient in removing the proteins and other contamination (McClintock, 2008). Hence, repeated alcohol precipitations are often necessary before the extracted DNA can be used in downstream application. This protocol can also be used by the small laboratories which do not have many facilities.

In a nutshell, the conventional phenol-chloroform extraction method and salting out method with difference parameters is tested in order to produce a high quality and quantity of total genomic DNA from hair samples of Jamnapari goat. The difference of parameters in the

temperature and time for incubation are carried out to determine the most optimal parameter for each method.

2.4 Previous Studies in the Extraction of DNA from Hair Samples

A study had been done to evaluate the effectiveness of three different methods in extraction of DNA from human hair. There are Chelex method, ISOHAIR method and QIamp DNA mini kit. More than 150ng of DNA was extracted when using the ISOHAIR method, while 120-140ng of DNA was extracted by using the Chelex method. The yield of DNA extracted with the QIamp DNA mini kit was quite low, which is less than 120ng and this method was relatively expensive. Although the ISOHAIR method could isolated more DNA, the products contains of PCR inhibitors, such as melanin species. Of the two methods, the Chelex method is recommended for DNA extraction from hair samples as it is free of the PCR inhibitor and cost-effectiveness. (Suenaga and Nakamura, 2005)

Due to low quantities and quality of DNA in hair samples, Bauerova, Bauer and Vasˇícˇek (1999) had done a study to focus on the application of more efficient DNA extraction methods on dog hairs. The t-test assessed that the mean of standard method was 32.04 ng DNA per 100 dog hairs, while the mean of Ca^{2+} improved method was 84.14 ng per 100 dog hairs. Concerning to the result, it is possible to obtain an increase of at least 100% with the new Ca^{2+} improved extraction method compared to the established method (Pfeiffer, Volkel, Taubert and Brenig, 2004) Besides that, the quality of the DNA from the hair sample must also be improved by removing the impurities. Bauerova, Bauer and Vasˇícˇek (1999) proved that the DNA purification by silica is effective as Prep-A-gene purification. In this

study, the silica matrix was recommended to purify the hair root DNA as this method ias an inexpensive alternative to Prep-A-gene purification.

On the other hand, Takayanagi, Asamura, Tsukada, Ota Saito and Fukushima (2003) carried out a study to investigate three DNA extraction methods from hair shafts, such as the phenol/chloroform method, NaI treatment method and silica-beads method. As a result of the extraction from different length of fresh hair shafts, the fluorescent peak heights as DNA recovery by three methods were sufficiently high. These methods are effective in isolating DNA from fresh hair shafts of 1-10 cm lengths. The phenol/chloroform methods needs a degree of technical proficiency, while the NaI method offers a stable extraction procedure and the silica-beads method offers the advantage of short extraction time and simple procedure.

Moreover, Nozawa *et al.* (1999) carried out the typing of nuclear DNA loci (HLA_DQA1) by using the cetyl-trimethyl ammonium bromide (CTAB) precipitation for DNA purification and a sensitive semi-nested PCR. This study proved that the isolation of DNA from 5 cm or 10 cm hair shaft invariably yielded good amounts of PCR products, while the 2.5 cm and 20 cm hair shaft failed to give any PCR product. Although one of specimens showed predominant appearance of one allele, all the genotypes of HLA_DQA1 were successfully determined from six known heterozygotes,

2.5 Polymerase Chain Reaction

Polymerase chain reaction (PCR) is enzymatic process where millions of DNA fragment can be synthesized by using the oligonucleotide primers that flank the DNA template (Hochmeister, 1995).

The PCR is based on three process- denaturation, annealing and extension by DNA polymerase. Denaturation is carried out by heating the sample to about 95°C - 98 °C in order to separate the double stranded DNA into complementary single strand. During the annealing process, the synthetic oligonucleotide primers and short sequences of nucleotides bind to the complementary single strands. This primer annealing is generally done by lowering the temperature to between 40 °C and 60 °C. Then, the *Thermus Aquaticus* (Taq) are used to extend the primer and synthesized the new DNA strands that are complementary to the DNA template. These three steps represent a single PCR cycle. When this process is repeated for 25-30 cycles, millions of copies of target sequences can be obtained. (Smith, 2009)

The advantage of using PCR is the amplification of DNA possible even the concentration of DNA is low. This is vital in order to allow the genetic information to be collected from samples that have insufficient cellular material for other genotyping approach likes the shed hairs. Besides that, this process is very simple and result can be obtained in a short time. Moreover, PCR can also be used in amplification of degraded DNA (Sensabaugh and Beroldingen, 1991). Hence, the application of polymerase chain reaction is very important in forensic, genetic engineering, environment monitoring and so on.

2.6 *Cytochrome b*

During polymerase chain reaction (PCR), *cytochrome b* has been used as a primer to amplify the specific region of the DNA. The *cytochrome b* is one of the most widely used genes, which involve in the electron transport for the respiratory chain of mitochondria (Chen *et al.*, 2009). The mitochondrial *cytochrome b* is a membrane protein and the central component of cellular energy conservation machinery in different organisms. It is used to

translocate the protons across the membranes for ATP synthesis and various cellular processes. (Chen *et al.*, 2009)

Cytochrome b gene is widely used for the phylogenetic studies. The wide use of this gene has created a status as a universal metric, in the sense that studies can be easily compared. It is highly variable and conserve within the population level, which can be used for clarifying deeper phylogenetic relationship. However, the cytochrome *b* gene is under strong evolutionary constraints because some parts of the gene are more conserved than others due to functional restrictions (Meyer 1994).

Lee *et al.* (2008) carried out a study to identify the avian species by using the *cytochrome b* gene, where a series of primer pairs producing amplicons of decreasing size was designed. A total of 331 avian samples that represent 40 species were tested. Sequencing of the amplicons revealed limited intraspecies variation and no DNA sequences was shared by samples from two different avian species. The closest genetic distance among the 40 species was 0.059 which was between *Lonchura punctulata* and *Estrilda melpoda* based upon data from the smallest amplicon.

2.7 Agarose Gel Electrophoresis

The electrophoretic separation of an aliquot of the extract is one of the important methods for the determination of the quality and quantity of extracted DNA. The negatively charged DNA fragments are migrated to the anode and their distance depend on the size of DNA fragments, agarose concentration, usage of voltage, buffer and so on (Maniatis *et al.*, 1989). Linear double stranded DNA move through the gel matrix at a rate that is inversely proportional to the $\log_{10}$ of the number of base pairs. Hence, the fragment length can be seen

from the migration distance measured from the start point. (Hochmeister, 1995) In this method, a small portion of each DNA samples and quantitation standard are "loaded" into the wells of gel. The DNA is stained and visualized by using UV illumination or images are generated through the computer software. The undegraded DNA normally will appear as a compact band near the origin band, which is similar with the standard control, while the degraded DNA will form a smear throughout the gel by depending to the various sizes of DNA fragments. (McClintock, 2008)

2.8 Quantification by UV Spectrophotometry

UV spectrophotometer is used to determine the quantity and purity of isolated DNA through the measurement of the light absorption by the DNA solution. The absorption peak of extracted DNA is about 260 nm where allows to estimate the concentration of DNA, while the phenol and protein absorption peak is at about 280 nm. The isolated DNA, which is free from the phenol or protein, has approximately 1.8 for A260/A280. (Hochmeister, 1995) Strong absorbance around 230nm can indicate that organic compounds or chaotropic salts are present in the purified DNA. A ratio of 260nm to 230nm can help evaluate the level of salt carryover in the purified DNA. The A_{260}/A_{230} is best if greater than 1.5.

Haque *et al.* (2003) carried out study to evaluate the performance of spectrophotometric (OD) DNA quantification and the fluorometric quantification methods. The PicoGreen assay (PG) and a novel real time quantitative genomic PCR assay (QG) that specific to the region of human *BRCA1* locus revealed that the OD method was most precise and concordant with the reference sample. The absorbance of spectrophotometer for DNA was less biased than the fluorometric method which are rely to the multiple interacting component and more indirect measurement of DNA concentration.

3.0 Materials and Methods

3.1 Preparation of hair samples

Hair samples were plucked from 5 different Jamnapari goats. Then, the hair samples were washed with the distilled water and 70% ethanol. After washing, it was transferred into falcon tube.

3.2 Conventional Phenol-chloroform extraction method

The extraction of DNA from the hair samples were done according to the procedure of Heywood *et al.* (2002) with some modification. DNA was extracted from 10 and 50 hairs per goat by cutting approximately 1 cm of the root end of each hair with a new sterile blade and placed in a 1.5 ml microcentrifuge tube. 500 µl of lysis buffer (10 mM Tris-HCL, pH8.0; 25 mM EDTA; 100 mM NaCl; 0.5% SDS) and 25 µl proteinase K (20µg/µl) were added. Then, the solution was incubated at different temperatures and times:

Table 2: The temperatures and times for incubation in phenol chloroform method

Temperature (°C)	Time (hour)
37	18
55	5
55	18

The digested hair solution was then extracted by adding equal volume of phenol: chloroform (1:1) and was centrifuged for 15 minutes at 13000 rpm. Then, the aqueous phase was transferred into a new 1.5 microcentrifuge tube. The resulting DNA in the final aqueous

phase was then precipitated using an equal volume of absolute ethanol (-20°C) and 1/10 volume of 3 M sodium acetate buffer (pH 6). The DNA was pelleted by centrifugation at 13000 rpm for 10 minutes and the pellet was then washed with 70% ethanol. After that, the ethanol was slowly discarded and the tube was allowed to air dry at room temperature for 30 to 60 minutes. Lastly, the pellet was resuspended in 40µl of nuclease free solution and stored at -20 °C for further analysis. Gloves were used all the times to prevent DNA from the hands contaminating samples.

3.3 Salting out method

The isolation of DNA from the hair samples of Jamnapari goats were carried out according to the procedure of Mohammadi and Saberivand (2009) with some modification. DNA was extracted from 10 and 50 hairs per goat by cutting approximately 1 cm of the root end of each hair with a new sterile blade and placed in a 1.5 ml microcentrifuge tube. 500 µl of lysis buffer (10 mM Tris-HCL, pH8.0; 25 mM EDTA, pH8; 100 mM NaCl; 0.5% SDS) and 25 µl proteinase K (20µg/µl) were added. Then, the solution was incubated at different temperatures and times:

Table 3: The temperatures and times for incubation in salting out method

Temperature (°C)	Time (hour)
37	18
55	5
55	18

The digested hair solution was then extracted by adding equal volume of saturated NaCl (6M) and was centrifuged for 15 minutes at 13000 rpm. Then, the aqueous phase was transferred into a new 1.5 microcentrifuge tube. The resulting DNA in the final aqueous phase was then precipitated using an equal volume of absolute ethanol (-20°C) and 1/10 volume of 3 M sodium acetate buffer (pH 6). The DNA was pelleted by centrifugation at 13000 rpm for 10 minutes and the pellet was then washed with 70% ethanol. After that, the ethanol was slowly discarded and the tube was allowed to air dry at room temperature for 30 to 60 minutes. Lastly, the pellet was resuspended in 40µl of nuclease free solution and stored at -20 °C for further analysis. Gloves were be used all the times to prevent DNA from the hands contaminating samples.

3.4 Agarose gel electrophoresis

The quality and quantity of isolated DNA were checked using agarose gel electrophoresis. It is also used for determining the presence of DNA molecules. For a 1% agarose gel, 0.4 g of agarose was weighed out into a flask and 40ml of 1 x TAE buffer was added. The suspension was then heated in a microwave for 1 minute in order to dissolve the agarose powder. After heating, approximately 0.8ul of ethidium bromide was added to the solution and was allowed to cool. In the mean time, the gel casting tray was prepared by sealing ends of gel chamber with tape or appropriate casting system. Appropriate number of combs was placed in gel tray. Then, the solution was poured into gel tray. It was then allowed to solidify in the room temperature. After that, combs were removed and the gel was covered with TAE buffer. Loading buffer was added to samples. DNA and standard DNA (Ladder) were loaded onto gel. The agarose gel electrophoresis was done at 100V for 25 minutes. DNA bands were visualised using UV lightbox or gel imaging system.